



(1) Publication number:

0 224 287

A1

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 86201878.5

(22) Date of filing: 28.10.86

(51) Int. Cl.*: C 12 N 15/00 A 01 H 1/00, C 12 N 5/00 C 12 N 1/20, C 12 P 21/02 //(C12N1/20, C12R1:41), (C12N1/20, C12R1:01)

(30) Priority: 29.10.85 NL 8502948

Date of publication of application: 03.06.87 Bulletin 87/23

Designated Contracting States:
 AT BE CH DE FR GB IT LI LU NL SE

(1) Applicant: Rijksuniversiteit Leiden Rapenburg 73 Leiden(NL)

(7) Inventor: Schilperoort, Robbert Adriaan Antonie Duycklaan 10c NL-2334 CD Leiden(NL)

(7) Inventor: Hooykaas, Paul Jan Jacob Floris Versterlaan 12 NL-2343 RS Oegtgeest(NL)

(72) Inventor: Hoekema, Andreas 141 Sanfrancisco St. App. 3 CA 94005 Brisbane(US)

(72) Inventor: van Veen, Ronald Jacques Maria Koestraat 15a NL-2312 XH Leiden(NL)

(72) Inventor: den Dulk-Ras, Harmke Gerard Brandstraat 24 NL-2332 AL Leiden(NL)

(74) Representative: van der Saag, Johannes et al,
OCTROOIBUREAU VRIESENDORP & GAADE P.O. Box 266
NL-2501 AW The Hague(NL)

(54) A process for the incorporation of foreign DNA into the genome of dicotyledonous plants.

(5) A process is disclosed for the incorporation of foreign , DNA into the genome of dicotyledonous plants comprising infecting these plants or incubating dicotyledonous plant protoplasts with bacteria suitable or made suitable for that purpose, which are provided with one or more tumour-inducing plasmids or derivatives therefrom, originally originating from Agrobacterium, or from bacteria which contain the T-DNA originating from the above-meant plasmids, and/or the virulence genes originating from the above-mentioned plasmids, incorporated elsewhere in the bacterial DNA.

CLAIMS

- 1. A process for the incorporation of foreign DNA into the genome of plants, by infecting these plants or explants from them, or incubating the plant protoplasts or cells with bacteria suitable or made suitable for that purpose, characterized in that dicotyledonous plants are infected or dicotyledonous plant protoplasts are incubated with bacteria suitable or made suitable for that purpose, which are provided with one or more tumour-inducing plasmids or derivatives therefrom, originally originating from Agrobacterium, or from bacteria which contain the T-DNA originating from the above-meant plasmids, and/or the virulence genes originating from the above-mentioned plasmids, incorporated elsewhere in the bacterial DNA.
- 15 2. A process according to claim 1, characterized in that for the infection or incubation use is made of Rhizobium bacteria or Phyllobacterium bacteria.
- 3. A process according to claim 1 or 2, characterized in 20 that bacteria are applied which are provided with one or more Ti- or Ri-plasmids or derivatives therefrom.
 - 4. A process according to claim 3, characterized in that

the bacteria used have been provided with a stable cointegrate plasmid, constructed from a plasmid R772 and a plasmid pTiB6 with foreign DNA incorporated in the T-region of the latter.

5. A process according to any of the preceding claims, characterized in that bacteria are used, which contain at least one plasmid, which has the Vir-region of a tumour-inducing plasmid but no T-region, and at least one other plasmid, which has a T-region with incorporated therein foreign DNA but no Vir-region.

- 6. Dicotyledonous plants and plant cells obtained after, applying the process according to any of the preceding 15 claims, the generic properties of the original plants or plant cells have been changed.
- 7. A process for the preparation of chemical and/or pharmaceutical products, characterized in that cells
 20 obtained with application of the process according to any of the claims 1-5 are cultivated and the desirable substance is isolated.
- 8. A process according to claim 7, characterized in that 25 culturing is effected by means of fermentation and if useful subsequent immobilisation.
- 9. A process according to any of the claims 1-5 incl. or 8, characterized in that the regulator regions positions 30 before and behind thd protein coding regions of T-DNA genes, in particular the genes for octopine synthesis for expressing foreign genes in dicotyledonous plant

cells are used.

- 10. Dicotyledonous DNA having a portion artificially inserted in it with the process according to any of the 5 preceding claims.
 - 11. Cell lines and regenerated plants obtained after application of the process according to any of the claims 1-9.

10

- 12. Rhizobium trifolii LPR 5087 and mutants thereof.
- 13. Phyllobacterium LAZ100 and mutants thereof.